Molecular Ecology Resources (2009) **9**, 551–554 doi: 10.1111/j.1755-0998.2008.02364.x

PERMANENT GENETIC RESOURCES NOTE

Development of nine new microsatellite loci for the American beaver, *Castor canadensis* (Rodentia: Castoridae), and cross-species amplification in the European beaver, *Castor fiber*

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Abstract

We developed nine new nuclear dinucleotide microsatellite loci for *Castor canadensis*. All loci were polymorphic, except for one. The number of alleles ranged from two to four and from five to 12 in populations from Arizona and Wisconsin, respectively. Average heterozygosity ranged from 0.13 to 0.86 per locus. Since cross-species amplification in *Castor fiber* was successful only in four loci, we tested also nine recently published *C. canadensis* loci in the Eurasian species. Eight of the published loci amplified; however, three were monomorphic. The number of alleles was lower in *C. fiber* than in *C. canadensis* at all loci tested.

Keywords: beaver, *Castor canadensis*, *Castor fibre*, cross-species amplification success, microsatellite

Received 31 May 2008; revision accepted 28 July 2008

The American beaver (*Castor canadensis*) is broadly distributed throughout North America and is considered to be an ecosystem engineer because it modifies surrounding habitat (Wright *et al*. 2002; Müller-Schwarze & Sun 2003). Ponds created by beaver can increase the number of species of herbaceous plants, provide habitat for waterfowl broods and fish reproduction (Naiman *et al*. 1988; Nummi 1992; Schlosser 1995; Barnes & Mallik 2001). However, alterations that beavers cause to their environment also can have negative economic impacts due to flooding (Müller-Schwarze & Sun 2003) and habitat modification. As a consequence, translocations and lethal methods have been used to control beaver populations. In addition, beavers have been hunted for their pelts since the early 1800s, and in many regions of North America, they were virtually extirpated by 1900 (Müller-Schwarze & Sun 2003). Although there have been many studies of beaver ecology, little is known about the effects of hunting and management plans on the genetics of this mammal (Müller-Schwarze & Sun 2003). The number of microsatellite loci available is limited to 10 in the American beaver (Crawford *et al*. 2008) and no loci have been described so far in the European sister species, *Castor fiber*. Therefore, we developed and characterized microsatellite DNA markers to investigate population structure, connectivity, and dispersal patterns of *C. canadensis* populations. We also tested the cross-species amplification success of the published and the newly developed American beaver loci in the European beaver.

We constructed a genomic library following the protocol described by Glenn & Schable (2005). Genomic DNA was extracted using the DNeasy tissue kit (QIAGEN) from 1 cm^3 of tissue collected from beaver tails from Tres Rios Wetlands in Phoenix, Arizona (AZ). The DNA was partially restricted with the enzyme RSAI (NEB) and fragments were ligated (using T4 DNA ligase) to double-stranded SNX-24 linkers (Glenn & Schable 2005). To create a whole genome PCR library, linker-ligated fragments were amplified by polymerase chain reaction (PCR) using an SNX-24 forward primer and high-fidelity DNA polymerase (Invitrogen).

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Table 1 Characteristics of the nine microsatellite loci developed and optimized for *Castor canadensis*. Locus name, GenBank Accession number, primer sequences, cloned repeat, magnesium chloride concentration, Annealing temperature (T_a), size of cloned repeat, mean number of alleles per locus (N_A), observed (H_O), and expected (H_E) heterozygosities are reported. TDN indicates the use of touchdown protocol

							C. canadensis							
							Tres Rios, Arizona (n = 57)				Wisconsin (n = 30)			
Locus	GenBank Accession no.	Primer sequence (5′–3′) F, forward; R, reverse	Repeat motif	Mg_2Cl (mM)	T_a (°C)	Size (bp)	Allelic range	N_A	H_O	H_E	Allelic range	N_A	H_O	H_E
Cca15	EU703999	F:/PET/AAGTGACTTTGGGCATTAACC R: GGACACCTGGTGCAATCC	$(GT)_{21}ATGTAT(GT)_3$	3.0	TDN	249	241–243	2	0.43	0.47	235–249	7	**0.13**	0.69*
Cca20	EU704000	F:/VIC/CCTCTGGGATCTGCACTGTC R: GTGGTTCAAGTGCAAGCAC	$(CA)_{14}$	3.0	TDN	305	303–309	2	0.42	0.48	303–319	7	**0.23**	0.78*
Cca56	EU704002	F:/FAM/GCAGAGCACCAATAAAATCC R: CTGGACTCTTGGAAACGCC	$(CA)_9TATA(CA)_{19}$	3.2	54	262	218–256	4	0.65	0.65	224–250	5	**0.36**	0.33
Cca62	EU704003	F:/NED/GCAAAAGGATTAGAATAACCAAGTG R: CCAGGGTGGATGAATTTG	$(GT)_3AGTTGCC(GT)_{11}$	2.5	TDN	329	341–349	3	0.63	0.63	303–353	7	0.46	0.82*
Cca76	EU704004	F:PET/CCCATAGAACCCAAAGCAG R: CAACTGACTGCAAATAGCTACAGC	$(GT)_{20}$	2.5	52	173	154–172	2	0.40	0.41	149–189	12	0.56	0.78*
Cca92	EU704005	F:/NED/TCCTTGACTCTGGGCATG R: CAGAGCTTCCACAGTATCTGG	$(CA)_{20}$	3.5	TDN	204	198–202	3	0.54	0.65	196–208	6	**0.56**	0.76*
Cca112	EU704006	F:/VIC/CCCAAAAAAGATGTTCTT R: GACTTTGCTGGTTTAGAGGTTG	$(GT)_{18}$	3.2	55	198	192–194	2	0.27	0.24	186–204	7	0.86	0.75
Cca122	EU704007	F:/FAM/ACCAAGTGAATTAACATACCCATC R: CAAGTATACAGTTGTCAGCATGTTAC	$(GT)_{22}$	3.5	TDN	176	164–172	3	0.65	0.62	154–188	9	0.3	0.85*

*locus not in Hardy–Weinberg equilibrium ($P < 0.05$).
H_O in bold indicates the likely presence of null alleles.

This library was hybridized to biotinylated microsatellite oligonucleotide probes $(GT)_{15}$ $(CT)_{15}$ and $(GATA)_8$. Hybridized fragments were captured on streptavidin-coated paramagnetic beads (Dynal). Microsatellite-enriched fragments were recovered by PCR and products were ligated and transformed using a TOPO TA cloning kit (Invitrogen). Approximately 270 colonies were amplified using M13 forward and reverse primers. We selected 200 nonredundant clones ranging from 500 to 1200 bp, as visualized on 1.5% agarose gels. Clones were sequenced using M13 primers on an ABI 3730xl genetic analyser (PE Applied Biosystems) using BigDye Terminator. Ninety clones had recognizable microsatellite sequences, of which 44% (40) had adequate flanking regions to design primers. Forty primer pairs were designed using Primer 3 software (Rozen & Skaletsky 2000). The designed primer pairs were double-checked for homodimers, hairpins and heterodimers using Oligo Analyser software (Integrated DNA Technologies; http://www.idtdna.com/analyser/Applications/OligoAnalyser).

Of the initial 40 primer pairs designed, 31 failed to amplify, one locus was monomorphic (Cca 151), and eight loci amplified and were variable (Table 1). We assessed variation among 55 individuals from the Tres Rios population and 30 individuals from north-central Wisconsin (WI). M13 primer sequences were added to the 5′ end of each forward primer (Schuelke 2000), and were used in combination with M13 fluorescently labelled primers (Table 1). PCR was performed in 15 μL volumes using 50–100 ng of genomic DNA, 1× PCR buffer (20 mM Tris-HCL, pH 8.4, 500 mM KCl, Invitrogen), 0.2 mM of each dNTP, 0.2 μM of each primer, 1 U of *Taq* DNA polymerase (Invitrogen), and a locus-specific conditions (Table 1). All PCRs were conducted using a Mastercycler Gradient (Eppendorf) and the thermal profile for loci Cca56, Cca76 and Cca112 was an initial denaturation at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 30 s at the locus-specific annealing temperature (Table 1) and 72 °C for 30 s, followed by a final extension of 3 min at 72 °C. The rest of the loci were amplified using a touchdown protocol that consisted of an initial denaturation at 94 °C for 2 min followed by three cycles at 94 °C for 30 s, annealing at 60–50 °C for 30 s (2 °C decrease every three cycles), extension at 72 °C for 30 s, followed by 30 cycles at 94 °C for 30 s, 90 s at 50 °C and 72 °C for 30 s, followed by a final extension for 2 min at 72 °C. All PCR products were analysed using an ABI 3730 DNA Analyser with Gene Scan-500 LIZ size standard (Applied Biosystems).

We used the Genotyper (ABI) software to determine allele size, and genotypic disequilibrium between pairs of loci was tested using FSTAT 2.9.3 (Goudet 2001) and GenePop 1.2 (Raymond & Rousset 1995). Other population genetic parameters were estimated in Arlequin (Excoffier *et al*. 2005) and loci were tested for null alleles using Micro-Checker (Van Oosterhout *et al*. 2004). No linkage disequilibrium was observed in either the AZ or WI population after Bonferroni correction ($\alpha = 0.05$, $P > 0.05$; Rice 1989). The number of alleles ranged from two to four per locus in the AZ population and from five to 12 in WI (Table 1). Average heterozygosity ranged from 0.27 to 0.65 in the AZ population and from 0.13 to 0.86 in WI. No significant deviations from Hardy–Weinberg equilibrium (HWE) were observed in the AZ population. However, six loci (Table 1) showed significant deviations from HWE ($\alpha = 0.05$, $P > 0.05$; Rice 1989) for the WI individuals due to a deficiency of heterozygotes, which may be due to the Wahlund effect (Templeton 2006; Table 1). No loci in the AZ population showed evidence of null alleles (all $P > 0.05$) but four loci in the WI individuals may have null alleles (Cca15 $P < 0.001$, Cca20 $P < 0.001$, Cca56 $P < 0.01$ and Cca92 $P < 0.01$). The frequency of null alleles at these loci was 0.66, 0.31, 0.33, and 0.11, respectively, as estimated by the method of Chakraborty *et al*. (1992) performed on Micro-Checker.

It is known from mitochondrial DNA analyses (A.J. Piaggio *et al*., unpublished) that the AZ population was established by closely related individuals, which could explain the relatively low polymorphism observed. The WI samples demonstrate that the new markers developed have reasonable diversity in other populations.

Cross-species amplification was performed for these primers and the Crawford *et al*. (2008) primers in the Eurasian beaver (*C. fiber*). Four newly developed and eight previously published loci successfully amplified (Table 2); all newly developed loci and five previously published loci were polymorphic in 10 *C. fiber* individuals.

The development of these markers, and their cross-species utility, will allow population-level studies to be conducted to allow better management of these species and potentially allow for comparative studies between the species.

Table 2 Cross-species amplifications of the *Castor canadensis* loci in *Castor fiber*, including the number of individuals genotyped (*N*) and the number of alleles (N_A)

Locus	Allele sizes (bp)	*N*	N_A	*N* Crawford *et al*. (2008)	N_A Crawford *et al*. (2008)
Cca56	242–250	10	4		
Cca76	157–189	10	3		
Cca92	196–214	10	5		
Cca11	200–204	10	3		
Cca4*	384–396	57	6	60	10
Cca5*	145–166	57	4	60	11
Cca8*	380–404	57	7	60	10
Cca9*	117	57	1	60	10
Cca10*	112	57	1	60	13
Cca13*	264–272	57	4	60	6
Cca15*	193	57	1	60	5
Cca18*	217–219	57	2	60	5

*loci described in Crawford *et al*. (2008).

Acknowledgements

We thank David Bergman and Dale Nolte for helping coordinate this project. This project was funded through the generous support of the Tres Rios Wetlands, Phoenix Arizona. We are grateful to Roger E Joos and Patrick Viehoever for field assistance and to Tony Dee, Judith Ramirez, and Angela Cora Varas for expert technical assistance. We also would like to thank Julia Figueroa for laboratory support. Pavel Munclinger and Adam Pártl are supported by the Ministry of Education, Youth and Sport of the Czech Republic, MSMT grant 0021620828. The collection of European beaver samples was supported by grants of the Ministry of Environment of the Czech Republic VaV/620/1/03 and SP/2d4/52/07.

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